Tools for near-atomic resolution in single-particle cryogenic electron microscopy

VALORIZATION

Relevance

Cryogenic electron microscopy is a rapidly growing and powerful technique for elucidating the structure of biological macromolecules. It is becoming a popular technique in the field of structural biology, and is now used in structure-based drug design and vaccine development. The aim of our research was to develop, test and optimize novel methodologies for a reliable single-particle analysis of cryogenic electron microscopy data. Our reference-free approach improves the effectiveness and speed of the high-resolution single-particle analysis. Thus, our results hold great promise in the battle against some of the world's major healthcare problems thus potentially saving numerous human lives.

In Chapter 4, we challenged the results of (Mao et al. 2012; Mao et al. 2013b), which we find to be the result of manipulative data processing. Our studies triggered serious discussions in the fields of immunology, structural biology, medical biology, and about the functioning of the academic press (Cohen 2013; Henderson 2013; Mao et al. 2013a; Subramaniam 2013; van Heel 2013). This discussion is still ongoing, and has stimulated different parties in cryo-EM to pay attention to the issue of validation of the results. In particular, use of reference-free image processing. Thus, Electron Microscopy Database (EMDB; EBI-EMBL) now stimulates and demands a more detailed description of the processing details. A number of new online validation tools have been made available and a new database was initiated to make large raw datasets publically accessible. Moreover, this controversy opened up fundamental issues, related to the journal and referee responsibilities in the academic press. We propose further open discussions of the controversial results; transparency and open access to the original published data.

Target groups

The results of the work, presented in this thesis might be interesting to:

- research institutions, applying or starting to apply the cryo-EM methodologies in solving the structures of biological complexes
- pharmaceutical companies using structure-based drug design and vaccine development
- companies, developing and producing cryo-EM equipment, particularly electron microscopes, electron detectors and operating software
- manufacturers of various digital detectors for photography, medical imaging or astronomy
- academic press; funds, sponsoring HIV research and journalists, covering controversies in science and all the people interested in the development of the HIV vaccines

Activities/Products

Our methodological developments had or have been integrated into the popular scientific software package IMAGIC-4D (Image Science Software GmbH). This software pioneered many aspects of single-particle cryo-EM image processing and has been continuously developed for more than 35 years. The software is distributed semi-commercially on the basis of its maintenance costs.

Our *a posteriori* camera normalization, can be applied in various other fields of digital image processing, including photography, astronomy and medical imaging. The ideas are, however, simple and are thus easy to implement in any extensive image processing system.

The first cryo-EM structure of the worm hemoglobin, solved to near-atomic resolution, opens up new perspectives for studies of the process of the worm-hemoglobin oxygen binding, which in a long-term perspective could contribute to the development of the artificial blood.

Our cryo-EM study of the EspB protein open up new insight into the organization of the type VII secretion systems of *Mycobacteria* and contributes to our understanding of its virulence. This knowledge might facilitate the development of the new vaccine against *Mycobacterium tuberculosis*.

Innovation

In our work we suggested innovative ideas and tools in single-particle cryo-EM image processing (like a posteriori camera correction, P-spectrum, Fourier-space classification etc.). Apart from the theoretical and ideological considerations covered in the main text, our suggested and implemented methodologies are practically different from the analogues. Our approach is absolutely reference-free and thus has a less chance for obtaining invalid and reference-biased results; it is cheap and fast in term of computational power (all the details in chapter 5).

Schedule & Implementation

The suggested methodologies have been implemented in our IMAGIC-4D software and used for processing of the real challenging cryo-EM data for obtaining a near-atomic resolution. The ideas have been or will be published in the open literature without restrictions.

References

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